

## ELISA Method for StarLink Corn in Corn Flour and Corn Meal

First approval October 17, 2001

### Objective

Cry9C protein in StarLink (SL) corn is an endotoxin produced from a gene derived from *Bacillus thuringiensis* (*Bt*). This method is a quantitative enzyme-linked immunosorbent assay (ELISA) determination of Cry9C protein, utilizing proprietary antibodies, from EnviroLogix, Inc. The method is calibrated to estimate the weight percent of modified corn in corn flour and corn meal.

### Apparatus

1. Distilled or deionized water.
2. Glass bottles or flasks, 1200 ml.
3. Centrifuge tubes, 50-ml polypropylene, with conical bottom.
4. Plastic tape or Parafilm.
5. Precision pipets, capable of delivering 100–1000  $\mu$ l.
6. Class A pipets, 10 ml.
7. Vortex mixer.
8. Timer.
9. Balance, capable of 0.01-g measurement.
10. Centrifuge, capable of 5000  $\times g$ .
11. Multichannel pipette, 100  $\mu$ l.
12. Reagent reservoirs for multichannel dispensing.
13. Automated plate washer, or wash bottle.
14. Microtiter plate reader, capable of reading absorbance at 450 nm, preferably with subtraction of 650-nm reading.
15. Graduated cylinders, 50 and 1000 ml.
16. Orbital plate shaker (optional).

### Reagents

Items 1–7 are available as Cry9C Plate Kit from EnviroLogix, Inc.

1. Coated strip wells and strip holder.
2. Grain extraction concentrate (20 $\times$ ).
3. Cry9C enzyme conjugate.
4. Wash buffer salts.
5. Substrate solution.
6. Stop solution.
7. Calibration standards:
  - a. Cry9C negative control.
  - b. 0.01% SL.
  - c. 0.04% SL.
  - d. 0.125% SL.

## **ELISA Method for StarLink Corn in Corn Flour and Corn Meal (continued)**

8. Grain extraction solution. Dilute 1 part grain extraction concentrate plus 19 parts deionized water. Mix well.

9. Wash buffer. Add 1 packet of wash buffer salts to 1 liter of distilled water. Mix well.

### **Procedure**

See Notes 1–7.

#### *Sample preparation*

This method calls for a small sample to be analyzed. It is essential that this sample be well mixed and representative of the larger bulk from which it is drawn. Thorough mixing and determination of an appropriate sampling plan are critical to the results of this testing and are the responsibility of the user.

#### *Sample extraction*

1. Weigh  $5.0 \pm 0.1$  g corn meal or flour into a 50-ml centrifuge tube.
2. Add 50 ml grain extraction solution (reagent 8) to corn flour samples or 37.5 ml grain extraction solution to corn meal samples. Mix vigorously and allow to extract without agitation for 1 hr. Mix again at the end of the 1 hr.
3. Centrifuge at  $5000 \times g$  for 5 min.

#### *Sample extract dilution*

Concentrations of Cry9C endotoxin in StarLink corn can vary greatly. If sample extracts produce more color than the highest calibrator, dilute the sample extract in grain extraction solution (reagent 8) and test again. Failure to sufficiently dilute extracts can result in erroneously low results.

#### *ELISA assay*

1. Remove coated strip wells and holder (reagent 1) from foil bag. Place unneeded strip wells in bag and reseal. Secure desired number of test strip wells in strip holder, allowing two wells per sample and per standards.
2. Add 100  $\mu$ l of negative control (reagent 7a), 100  $\mu$ l of each calibrator (0.01, 0.04, 0.125% SL, reagents 7b–d), and 100  $\mu$ l of each clarified sample extract to duplicate wells. Follow this same order of addition for all reagents.
3. Thoroughly mix contents of wells by moving strip holder in a rapid circular motion on benchtop for 20–30 sec. Be careful not to spill contents.
4. Cover wells with tape or Parafilm to prevent evaporation and incubate at ambient temperature for 30 min. If an orbital plate shaker is used, shake at 200 rpm.
5. Carefully remove covering and vigorously shake contents of wells into a sink or other suitable container. Flood the wells completely with wash buffer

## ELISA Method for StarLink Corn in Corn Flour and Corn Meal (continued)

(reagent 9), then shake to empty. Repeat this wash step three times. Alternatively, perform these four washes (300 µl per well) with a microtiter plate washer. Slap plate on a paper towel to remove as much water as possible.

6. Add 100 µl of Cry9C enzyme conjugate (reagent 3) to each well.

7. Thoroughly mix contents of wells as described in step 4.

8. Cover wells with new tape or Parafilm to prevent evaporation and incubate at ambient temperature for 2 hr. If an orbital plate shaker is used, shake at 200 rpm.

9. After incubation, wash wells with wash buffer as described in step 4.

10. Add 100 µl of substrate solution (reagent 5) to each well.

11. Thoroughly mix contents of wells, as in step 3. Cover wells with new tape or Parafilm and incubate for 30 min at ambient temperature. Use orbital shaker if available, as described above.

12. Add 100 µl of stop solution (reagent 6) to each well and mix thoroughly. Read plate within 30 min (450 nm minus 650 nm reference).

### Calculations

Perform quadratic least-squares regression of eight calibration data points (duplicate wells of negative control and the three non-zero-percent StarLink calibrators) to obtain calibration equation in the form:

$$\%SL = a(OD)^2 + b(OD) + c$$

where OD is optical density of sample well and  $a$ – $c$  are least squares coefficients. Calculate percent (%SL) for all sample wells. Report average of two wells as the result. If further dilution of a sample extract was required to place results within standard curve, multiply final %SL by appropriate dilution factor.

A run is accepted if *all* of the following criteria are met:

a. Average OD of negative control is 0.25.

b. Average OD of the 0.01% SL calibrator is 0.08.

c. The coefficient of variation of pairs of OD of 0.01, 0.04, and 0.125% SL standards is 15%.

d. The  $r^2$  correlation statistic is >0.995.

### Notes

Use the following general precautions:

1. Store all kit components at 4–8° when not in use.
2. Do not expose kit components to temperatures greater than 37° or less than 2°.
3. Allow all reagents to reach ambient temperature (18–27°) before use.
4. Do not use kit components after stated expiration date.

**ELISA Method for StarLink Corn in Corn Flour and Corn Meal  
(continued)**

5. Do not expose substrate solution to sunlight during pipetting or while incubating in test wells.
6. Do not dilute or adulterate test reagents or use samples not called for in test procedure.
7. Samples that cannot be extracted immediately may be stored frozen for up to 1 week before analysis.